PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P200201746	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/DK2004/000047	International filing date *(day/month/year)* 22.01.2004	Priority date *(day/month/year)* 22.01.2003
International Patent Classification (IPC) or national classification and IPC C07K17/00, G01N33/68, C12N11/00		
Applicant BIONANOPHOTONICS A/S et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 10 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.11.2004	26.04.2005
Name and mailing address of the International preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Schmidt, Harald Telephone No. +31 70 340-4023

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000047

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-43	as originally filed
Claims, Numbers	
1-58	filed with telefax on 23.11.2004
Drawings, Sheets	
1-20	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 1-58
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-29
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-29
Industrial applicability (IA)	Yes:	Claims	1-29
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Re Item I

Basis of the report

The amendments filed with the letter dated 23.11.2004 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following:

Claims 1-9,14-21:

The amended portion "wherein more than one disulfide bridge containing proteins are coupled" in independent claim 1 is not supported by the description as filed. Applicant cited passages on page 12,17 and 21; however, none of this passages deals with or even suggest the technical feature that the proteins or peptides contain more than one disulfide bridge.

Furthermore, the amendments cause the international application to be objectionable under the PCT by introducing inconciseness under Article 6 PCT in combination with Rule 6(1)(a) PCT, since claim 2 does not contain any additional technical feature with respect to claim 1.

Claim 10 is an amalgation of former claim 28 dealing with a prediction method with passages on description pages 15 and 16. However, the specific spatial proximity of 10 Å in previous claim 28 is replaced with the more general, non-allowable term "close spatial proximity".

The combination of a prediction method with a method of coupling are two completely different processes that are not allowed to be linked together.

Hence, the subject-matter of claim 10 is not considered to be supported by the application as filed.

Moreover, said amendment causes the international application to be objectionable under the PCT by introducing obscurity under Article 6 PCT, since the term "close" is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers.

Claims 22-30 refer to claims 1-21 which are not admissible under Article 34(2)(b) PCT.

Claims 31-46:

The amendments cause the international application to be objectionable under the PCT by

introducing either (1) obscurity or (2) non-unity, which is not allowable under Article 34(2)(b) PCT.
(1) Although claims 1 and 31 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought or in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness and as such do not meet the requirements of Article 6 PCT.

(2) If claims 1 and 31 remain independent, an objection against unity of invention (Rule 13 PCT) arises for the following reasons:
The technical feature "more than one disulfide bridge containing proteins or peptides are coupled" of claim 1 makes a contribution over the prior art D1 to D3 and can be considered as special technical feature within the meaning of Rule 13.2 PCT.
Since the special technical feature "more than one disulfide bridge containing proteins or peptides are coupled" of claim 1 is lacking as limiting technical feature in claim 31, and vice versa, the special technical feature "the carrier is capable of being decoupled from the protein by irradiation" of claim 31 is lacking as limiting technical feature in claim 1.

Concerning amended claim 32: see remarks on amended claim 2.

Claims 47-52 refer to claims 31-46 which are not admissible under Article 34(2)(b) PCT.

Claims 54-56:
The cited passage on page 20 refers to localized delivery of the molecule-coupled drug by implantation at the site of treatment. However, claim 54 generalizes the implantation to administration of the complex, which also includes an oral administration. Such a generalization is not supported by the specification and introduces subject-matter which extends beyond the content of the application as filed.

Claims 57 and 58 refer to claims 1-21 and 31-46 which are not admissible under Article 34(2)(b) PCT.

Since the amended set of claims is not allowable pursuant to Article 34(2)(b) PCT,

amended claims 1-58 are disregarded for the following items IV and V. Hence, the international preliminary examination report is based on the claims 1-29 as originally filed.

Re Item IV

Lack of unity of invention

This Authority considers that there are two inventions covered by the claims indicated as follows:

I: Claims 1-27 directed to methods of coupling a disulfide bridge containing protein or peptide to a carrier

II: Claims 28 and 29 directed to a method of predicting a disulfide bridge containing protein or peptide capable of disruption by irradiation

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

the technical feature of claim 1 resides in the step of coupling a disulfide bridge containing protein or peptide to a carrier. Neither the same nor a corresponding special technical feature is present in the method claim 28 dealing with a method of predicting a disulfide bridge containing protein or peptide capable of disruption by irradiation.
Furthermore, the prediction method is not a prerequisite for the coupling method of invention I. In the absence of any teaching as to the relationship between invention I and II, there is no single general inventive concept that links inventions I and II.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following document is referred to in this communication:

D1: WO 91/16425 A (SIGRIST HANS ;KLINGLER DABRAL VIBHUTI (DE); DOLDER MAX (CH); WEGMU) 31 October 1991 (1991-10-31)

D2: WO 94/01773 A (BIOMIRA INC ; QI PEI (CA); SYKES THOMAS R (CA); WOO THOMAS K (CA); NOU) 20 January 1994 (1994-01-20)

D3: NEVES-PETERSEN MT ET AL.: "High probability of disrupting a disulphide bridge mediated by an endogenous excited tryptophan residue" PROTEIN SCIENCE, vol. 11, 2002, pages 588-600, XP002288112

INDEPENDENT CLAIMS 1,16,24 AND 26

The document D1 discloses a method of coupling a peptide to a carrier by incubating the peptide with a support, followed by irradiating the peptide to bind to the modified surface (see pages 2, 3 and 5).
It is further taught that biomolecules may be bound to the surface through their thiol function (see page 2).
It is assumed that the method of D1 results in the creation of a thiol group in the protein by disulfide disruption. The irradiated protein is then capable of binding to the modified surface.
Applications for such carriers are in the field of e.g biosensors (see claims 2 to 6).

The document D2 discloses photoactivation of proteins by ultraviolet radiation, which enables reaction with other chemical entities to form conjugates. It is further taught that the effect of the radiation is the reductive cleavage of the disulfide bonds to leave reactive free thiol groups and that such a protein can react with any sulfhydryl reactive agent (see pages 1,11 and 19).

Therefore, subject-matter of independent claims 1,16,24 and 26 does not meet the requirements of Article 33(2) PCT.

INDEPENDENT CLAIM 28

The document D3 discloses a study on the probability of irradiating a disulfide bridge in a protein, using cutinase as a model protein.
It is further taught that tryptophan is in spatial vicinity of the SS bond and it is assumed that the plane of the dipole of the side-chain of tryptophan is not orthogonal to the plane of the disulfide bridge.
Therefore, subject-matter of claim 28 is not new within the meaning of Article 33(2) PCT.

DEPENDENT CLAIMS 2-15, 17-23, 25, 27 and 29

Dependent claims 2-15, 17-23, 25, 27 and 29 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step (Article 33(2) and (3) PCT).

Claims

1. A method of coupling a disulfide bridge containing protein or peptide to a carrier comprising the following steps,
   a) irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption, and
   b) incubating the irradiated protein or peptide with a carrier capable of binding a thiol group and thereby obtaining a coupling,
   or
   a) incubating the protein or peptide with a carrier capable of binding a thiol group, and
   b) irradiating the protein or peptide in the presence of said carrier to create a thiol group in the protein or peptide by disulfide bridge disruption and thereby obtaining a coupling

   wherein the carrier is an insoluble support and whereon more than one disulfide bridge containing proteins or peptides are coupled.
2. A method according to claim 1, wherein the protein or peptide comprises more than one disulfide bridge.
3. A method according to any one of claims 1 or 2, wherein said irradiation step comprises light of a wavelength that excites one or more aromatic amino acids.
4. A method according to any one of claims 1 to 3, wherein said irradiation step comprises light of a wavelength that excites one specific aromatic amino acid.

5. A method according to any one of claims 1 to 4, wherein said aromatic amino acid(s) is/are selected from tryptophan, tyrosine and phenylalanine.

6. A method according to any one of claims 1 to 5, wherein the irradiation is performed by multi-photon excitation, preferrably by two-photon excitation.

7. A method according to any one of claims 1 to 5, wherein said irradiation comprises light with a wavelength of about 295nm, 275nm or 254nm.

8. A method according to claim 5, wherein said aromatic amino acid is tryptophan.

9. A method according to claim 7, wherein the wavelength is about 295nm.

10. A method according to any one of claims 2 to 9, further comprising the steps of:

a) verifying one or more disulfide bridges in said protein or peptide,
b) identifying one or more aromatic amino acid residues in close spatial proximity to said one ore more disulfide bridges,
c) selecting a wavelength which specifically excites one or more of said aromatic amino acid residues, thereby disrupting one or more of said disulfide bonds.

11. A method according to claim 10 wherein the aromatic amino acid residue is within 10Å of the disulfide bridge

12. A method according to claim 11, wherein the plane of the dipole of the side-chain of the aromatic amino acid is not orthogonal to the plane of the disulfide bridge.

13. A method according to claims 10-12, wherein the amino acid residues located within an 8Å radius of the indole ring of said aromatic amino acid residue are over-represented by amidic amino acid residues (Asn, Gln), as well as, short aliphatic amino acid residues (Gly, Ala, Val) and /or long aliphatic amino acid residues (Leu, Ile) by at least 1 fold, and under-represented by charged amino acids (His, Lys, Arg)(Asp, Glu) and proline residues by at least 1 fold.

14. A method according to any one of claims 1 to 9, wherein said protein or peptide is irradiated in the presence of a free aromatic amino acid.

15. A method according to any one of claims 1 to 14, wherein said coupling is an immobilization on said support.

16. A method according to claim 15, wherein said immobilization is spatially controlled.

17. A method according to claim 16, wherein said support is a derivatised support that is capable of binding a thiol group.

18. A method according to claim 17, wherein said support comprises a thiol group or a disulfide bridge.

19. A method according to claim 18, wherein the support comprises a spacer.

20. A method according to any one of claims 1 to 19, wherein the protein or peptide can furthermore be released from the carrier by irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption.

21. A method according to claim 17, wherein said support comprises gold.

22. An insoluble support comprising one or more protein or peptide coupled by the method of any one of claims 1 to 21.

23. An insoluble support according to claim 22, wherein the coupled protein or peptide are specifically oriented.

24. An insoluble support according to claim 23, wherein the support is selected from the group consisting of an electronic chip, slide, wafer, resin, well, tube, microarray and membrane.

25. An insoluble support according to claim 24, wherein the support comprises a material selected from the group consisting of topaz, polystyrene, polyethylene, polyester, polyethermide, polypropylene, polycarbonate, polysulfone, polymethylmethacrylate, poly(vinylidene fluoride), silicone, diamond, quartz and silica, silicium, metal, nylon, nitrocellulose, agarose, cellulose and ceramic.

26. An insoluble support according to any one of claims 22 to 25, wherein the protein or peptide coupled to the support are spatially controlled.

27. Use of an insoluble support according to any one of claims 22 to 26 for a bio-functional reaction.

28. Use of an insoluble support according to claim 27, wherein said bio-functional reaction is selected from the group consisting of a bio-sensor, chromatography, immunodetection, enzyme assay, nucleotide binding detection, protein-protein interaction, protein modification, carrier targeting and protein targeting.

29. Use of the method according to any one of claims 1 to 21 for the production of a bio-sensor or a protein/peptide microarray.

30. Use of an insoluble support according any of claims 22 to 26 in a diagnostic or biosensor kit.

31. A method of coupling a disulfide bridge containing protein or peptide to a carrier comprising the following steps,

c) irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption, and

d) incubating the irradiated protein or peptide with a carrier capable of binding a thiol group and thereby obtaining a coupling,

or

c) incubating the protein or peptide with a carrier capable of binding a thiol group, and

d) irradiating the protein or peptide in the presence of said carrier to create a thiol group in the protein or peptide by disulfide bridge disruption and thereby obtaining a coupling

wherein the carrier is soluble and capable of being decoupled from said protein or peptide by irradiation.

32. A method according to claim 31, wherein the protein or peptide comprise more than one disulfide bridge.

33. A method according to claim 31 or 32, wherein said irradiation step comprises light of a wavelength that excites one or more aromatic amino acids.

34. A method according to any one of claims 31 or 33, wherein said irradiation step comprises light of a wavelength that excites one specific aromatic amino acid.

35. A method according to any one of claims 31 to 34, wherein said aromatic amino acid(s) is/are selected from tryptophan, tyrosine and phenylalanine.

36. A method according to any one of claims 31 to 34, wherein the irradiation is performed by multi-photon excitation, preferably by two-photon excitation.

37. A method according to any one of claims 31 to 34, wherein said irradiation comprises light with a wavelength of about 295nm, 275nm or 254nm.

38. A method according to claim 37, wherein said aromatic amino acid is tryptophan.

39. A method according to claim 37, wherein the wavelength is about 295nm.

40. A method according to any one of claims 31 to 39, further comprising the steps of:

a) verifying one or more disulfide bridges in said protein or peptide,

b) identifying one or more aromatic amino acid residues in close spatial proximity to said one ore more disulfide bridges,

c) selecting a wavelength which specifically excites one or more of said aromatic amino acid residues, thereby disrupting one or more of said disulfide bonds.

41. A method according to claim 40 wherein the aromatic amino acid residue is within 10Å of the disulfide bridge

42. A method according to claim 41, wherein the plane of the dipole of the side-chain of the aromatic amino acid is not orthogonal to the plane of the disulfide bridge.

43. A method according to claims 40- 42, wherein the amino acid residues located within an 8Å radius of the indole ring of said aromatic amino acid residue are over-represented by amidic amino acid residues (Asn, Gln), as well as, short aliphatic amino acid residues (Gly, Ala, Val) and /or long aliphatic amino acid residues (Leu, Ile) by at least 1 fold, and under-represented by charged amino acids (His, Lys, Arg)(Asp, Glu) and proline residues by at least 1 fold.

44. A method according to any one of claims 31 to 39, wherein said protein or peptide is irradiated in the presence of a free aromatic amino acid.

45. A method according to any one of claims 31 to 44, wherein the protein or peptide may be released from the carrier by irradiating the protein or peptide to create a thiol group in the protein or peptide by disulfide bridge disruption.

46. A method according to claim 31, wherein said carrier comprises a peptide, a protein or another biomolecule.

47. A coupled carrier coupled to one or more proteins or peptides obtainable by the method of any one of claims 31 to 46.

48. A carrier according to claim 45, wherein the coupled protein or peptide are specifically oriented.

49. A coupled carrier according to claim 47 or 48, wherein the one or more protein or peptide is selected from the group consisting of an enzyme, transcription factor, protein domain, binding protein, antigen and immunoglobulin.

50. A coupled carrier according to claim 49, wherein said immunoglobulin is a F(ab) fragment.

51. A coupled carrier according to claim 49, wherein said enzyme is selected from the group consisting of cutinase, chymosin, glucose oxidase, lipase, lysozyme, alkaline phosphatase and plasminogen,

52. A coupled carrier according to claim 47, wherein the protein or peptide comprises a drug or a prodrug.

53. Use of a coupled carrier according to any one of claims 47 to 52 for drug delivery

54. A method of delivering of a drug or prodrug to a patient comprising the following steps of:

(a) providing a carrier coupled to one or more proteins or peptides according to any one of claims 47 to 52

(b) administering the carrier-coupled protein or peptide to a patient

(c) irradiating the carrier-coupled protein or peptide to create a thiol group in the molecule by disulfide bridge disruption and thereby releasing the protein or peptide from the carrier.

55. A method according to claim 54, wherein the carrier is a pharmaceutical drug.

56. A method according to claim 54, wherein the protein or peptide is a drug or a prodrug.

57. A method of predicting a disulfide bridge containing protein or peptide capable of disruption by irradiation for use in the method of claims 1-21 or method of claims 31-46, comprising the steps of:

a) identifying and selecting a disulfide bridge containing protein or peptide, and

b) identifying and selecting a protein or peptide selected in (a), further comprising an aromatic amino acid residue within 10Å of said disulfide bridge, and

c) identifying and selecting a protein or peptide selected in (b), wherein the plane of the dipole of the side-chain of said aromatic amino acid is not orthogonal to the plane of said disulfide bridge.

58. A method according to claim 57, further comprising the step of: identifying and selecting a protein or peptide selected in (b) or (c), wherein the amino acid residues located within an 8Å radius of the indole ring of said aromatic amino acid residue are over-represented by amidic amino acid residues (Asn, Gln), as well as, short aliphatic amino acid residues (Gly, Ala, Val) and /or long aliphatic amino acid residues (Leu, Ile) by at least 1 fold, and under-represented by

charged amino acids (His, Lys, Arg)(Asp, Glu) and proline residues by at least 1 fold.